TRANSLATION

(19) **FEDERAL REPUBLIC OF GERMANY** [Seal]

(12) **Patent**

(10) **DE 100 60,036 C1**

(51) Int. Cl.[7]:
A 61 L 27/12
A 61 L 24/02
A 61 L 27/38

GERMAN PATENT AND TRADEMARK OFFICE

(21) Reference: 100 60 036.0-45
(22) Date filed: December 2, 2000
(43) Date laid open: --
(45) Date of publication of the patent granting: August 8, 2002

Opposition can be raised within 3 months after publication of the granting.

(73) Patent proprietor:
DOT GmbH, 18059 Rostock, DE

(72) Inventor:
Gerber, Thomas, Prof. Dr., 18059 Papendorf, DE

(74) Representative:
Schnick and colleagues, 18057 Rostock

(56) The following documents were taken into consideration for evaluating patentability:
DE 198 25,419
DE 299 22,585 U1
GB 2,348,872 A
US 5,549,123 A
US 6,013,591
WO 92 21,302 A1

(54) Inorganic resorbable bone replacement material

(57) The invention concerns an inorganic, resorbable bone replacement material based on calcium phosphates.

The material is characterized in that it possesses a loose crystal structure, i.e., the crystallites are not tightly assembled as in a solid (ceramics), but are joined together only by means of several groups of molecules. The volume, which is occupied by collagen in natural bone, is present in the material as interconnecting pores in the nanometer range. A second pore size, also interconnecting pores and in the range of several micrometers, makes possible an ingrowing of collagen fibers for forming tissue. These fibers are seeds for the biomineralization that is initiated (formation of the body's own biological apatite).

The material contains a third category of interconnecting pores, which are appropriate for spongy bone and these lie in the range of approximately 100 µm to 1000 µm and thus make possible an ingrowing of blood vessels, whereby the resorption and the new formation of bone result not only as a front of healthy bone, but take place throughout the entire defect.

The large inner surface area of the material makes it possible to bind the body's own growth factors or synthetic ones.

Description

[0001] The invention concerns an inorganic, resorbable bone replacement material based on calcium phosphates.

[0002] After application of blood components, the transplantation of bone is the second most frequent form of transplant in humans [1]. Thus in the USA in 1993, 250,000 bone transplants were conducted [2]. The replacement of post-traumatic bone defects (which occur as a consequence of osteomyelites and tumor operations) as well as osteoporotic bone defects is of significant clinical importance, since a rehabilitation inclusive of function is possible only in this way.

[0003] The method denoted the "gold standard", which is the removal of autologous bone, for the most part from the hip ridge, involves additional costs, risks, and stresses for the patient, and there are limits relative to the quantity of bone that is made available. The partially extensive defects that arise from this surgical removal are often painful for a very long time and there is an increased risk of infection. In order to avoid these problems, different alloplastic and allogenic materials were developed, but up until now, no clinically satisfactory results with these have been presented [3]. Prior methods of filling the defect or regenerating the tissue (tissue bank material, plastics, inorganic materials) have disadvantages and risks such as viral infections, fibrotic reaction in the surrounding tissue, avitality or lack of resorption.

[0004] The development of an innovative group of inorganic biomaterials as alternatives for autologous osteoplastics represents considerable progress, since a secondary operation with its increased costs, risks and pain can be avoided, and the disadvantages of other methods, such as, e.g., the transmission of diseases (HIV, hepatitis, encephalitis, among others) to the implant or serious immune reactions do not occur in principle. A significant improvement in quality of life results for the affected individual due to a shortening of the healing phase until the bone is capable of bearing a load.

[0005] The regeneration of bone tissue can tak place in three different ways: osteogenesis, osteo-induction and osteoconduction [4]. Osteoconduction indicates growth arising from the bone tissue that is present along a leading structure, while osteo-induction denotes a stimulation of differentiation of storage tissue cells to form osteoblasts. In contrast, osteogenesis represents a new formation of bone from vital, transplanted bone cells.

[0006] Resorbability is viewed as the essential requirement for a bone replacement material. Bone continually passes through phases of integration and disintegration, called remodeling. A bone replacement material will now participate in this remodelling and in this way will be replaced by natural bone within a certain time (depending on the size of the defect, approximately 12 months). The disintegration of natural bone is produced by osteoclasts. In an ideal bone replacement, the resorption will also be produced by osteoclasts, since the disintegration of the material is coupled to the new formation of bone in this way. All other resorption mechanisms ultimately pass through a resorptive inflammation, which always inhibits the new formation of tissue—particularly if the inflammation is too intense.

[0007] Bone is a "composite material" comprised of an inorganic, mineral component and an organic component (collagen).

[0008] The mineral is a biogenic hydroxyapatite (HA), a calcium phosphate. Pure HA has the structural formula $Ca_{10}(PO_4)_6(OH)_2$. Biogenic HA, in contrast, has several substitutions. Thus, Mg, F and Cl (< 1 wt.%) are found substituted for Ca, and CO_3 groups are found instead of PO_4 groups (5.8 wt.% in bone) [15]. The crystalline structure of minerals is hexagonal, wherein the lattice parameters extensively correspond to synthetic HA (deviations in the 3rd decimal place, angstrom region). The minerals arranged between the collagen fibers have a pronounced plate-like form. The mean dimensions are 45 nm x 30 nm x 3 nm.

Electron-microscopic investigations demonstrate that these involve single crystals with structural defects [16], probably caused by the named substitutions. The microstructure of the collagen-mineral composite can be described briefly as follows. Collagen fibrils are arranged into parallel bundles corresponding to the external load. These are mechanically reinforced by HA crystals arranged between the fibrils. The plates therefore lie flat on the fibrils, whereby the crystallographic c-axis of the minerals is oriented parallel to the longitudinal axis of the fibrils. The site of accumulation on the collagen fibers is determined by the hierarchical structure of the collagen (molecular procollagen (triple-helix) microfibrils. Procollagen molecules are assembled in parallel with a characteristic shift. In the longitudinal direction, 35-nm gaps are found between the procollagen molecules. Finally, a structure with a 64-nm period results [17]. Superstructures of variable complexity (cords, lamellar bone, reticular bone; for structural models, see [18], [19] und [20]) are formed from this basic structure by oriented assembly of the fibrils. The gaps between the procollagen molecules are viewed as the site of primary seeding [19].

[0009] For a bone replacement material, it is ideal that a pore structure exists, such as that present in spongy bone. That is, interconnecting pores of approximately 0.2 mm to 0.8 mm diameter must exist. It is possible in this way for blood vessels to grow into the material and thus the remodelling process is made possible only in this way.

[0010] Porous bioceramics comprised of tricalcium phosphate (TCP)/hydroxyapatite (HA) and TCP/monocalcium phosphate monohydrate (MCPM), both alone as well as in combination with BMP, as well as bone-marrow cells for osteoconduction and osteo-induktion are the subject of international animal experiment research [6-11]. The open-pore lattice-type structure of resorbable TCP/HA promotes regeneration [12]. There are indications that integration and regeneration in macroscopic HA ceramics take place by resorption, microfracture and renewed osteoconduction [13]. By combination with BMP (bone morphogenic protein [14]) or osteoprogenitor cells, another increase

of the regeneration potential could be achieved by additional osteoconduction.

[0011] As a bone replacement, a composite material comprised of organic and inorganic materials has been demonstrated to be unfavorable, since organic components that are foreign to the organism cause rejection reactions (immune reactions) or lead to undesired resorptive inflammation.

[0012] A number of porous ceramics as bone replacement have been described in the patent literature. In U.S. Patent 5,133,756 A; 1992, the ceramics are produced from spongy bovine bone and thus have the required pore structure. The total organic matrix is removed and the ceramic component is annealed at temperatures from 1100°C to 1500°C.

[0013] Another method (U.S. Patent 4,861,733 A; 1989) starts with the framework of natural corals and the calcium carbonate is converted to calcium phosphate in a hydrothermal process. The advantage of this method is that the pore structure (pore-size distribution, morphology) is ideal for the ingrowing of the bone tissue.

[0014] The decisive disadvantage of these ceramics is that they are not resorbable. For the described materials, this means that the bone tissue in fact grows into the pore structure in a well-defined [excellent] manner. The solid crystal structure of the ceramics, however, does not participate in the bone remodelling. Therefore, it remains a foreign body and influences the mechanical properties. Particularly during bone growth, this leads to inflammation in the transition from tissue to ceramics.

[0015] Resorbable ceramics based on tricalcium phosphate have been described (US 5,141,511 A, 1992). Here also, these involve a solid crystal structure which arises due to sintering processes. Pores are introduced into the material only in a size of the order of magnitude of spongy bone. Resorption takes place due to the

solubility of tricalcium phosphate. In this way, however, an increased concentration of ions occurs locally and leads to a resorptive inflammation.

[0016] Bioactive glasses are also offered as bone replacement material (US 6,054,400 A, 2000; US 5,658,332 A, 1997). The inorganic material is present here as a vitreous solid. Pores of the order of magnitude of spongy bone permit an ingrowing of the tissue. Smaller pores are not present in the material.

[0017] Glass ceramics are also offered as a bone replacement (US 5,981,412 A, 1999). They can be compared with bioactive glasses, wherein the calcium phosphate is present as a crystalline component in a glass matrix.

[0018] Calcium phosphate cements were also developed as another group of materials for application as a bone replacement (US 5,997,624 A, 1999; US 5,525,148 A, 1996). The decisive disadvantage of this group of materials is that defined, interconnecting pores are not introduced into the material, so that they are limited to very small bone defects.

[0019] It is generally established that hydroxyapatite ceramics or hydroxyapatite substances with the most varied pore structures are known, but that it is common to all of these that the capacity for resorption is lacking for the characteristic solid crystal structure or the crystal structure that is not described in detail.

[0020] US 5,549,123 A, which discloses, for example, a method for achieving a specific porosity, does not contain a description of the crystal structure; only in claim 8 is calcium phosphate described in very general terms.

[0021] For example, DE 299 22,585 U1 shows a filler for bone defects that is comprised of phase-pure β-tricalcium phosphate with two pore systems. The crystal structure is not described in detail as a decisive characteristic feature. As opposed to hydroxyapatite, β-tricalcium phosphate has the disadvantage that

there is an increased Ca-ion concentration due to its solubility and thus new formation of tissue is inhibited due to resorptive inflammation.

[0022] GB 2,348,872 A concerns a porous calcium phosphate substance with pores of 50 µm and up, which does not have the characteristic crystal structure of the present invention that is described later. The essential new properties of the material according to the invention are also not achieved in this way.

[0023] Further, reference is made to DE 198 25,419 A1 and US 6,013,591 A, wherein it is known to produce calcium phosphate ceramics with pores in the nanometer range. Here also, the decisive crystal structure is not described.

[0024] The object of the present invention, in contrast to this, is to supply a bone replacement material, which supports a formation of bone tissue (which is thus osteoconductive or osteo-inductive) and which is resorbed by the natural processes of bone remodelling.

[0025] The object is solved according to the invention by a material which possesses a loose crystal structure of calcium phosphates, i.e., the crystallites are not tightly assembled as in a solid (ceramics), but are joined together only by means of several groups of molecules. The volume, which is occupied by collagen in natural bone, is present in the material as interconnecting pores in the nanometer range. A second pore size, also of interconnecting pores and in the range of several micrometers, makes possible an ingrowing of collagen fibers for forming tissue. These fibers are seeds for the biomineralization that is initiated (formation of the body's own biological apatite). The material contains a third category of interconnecting pores, which are appropriate for spongy bone and thus lie in the range of approximately 100 µm to 1000 µm and thus make possible an ingrowing of blood vessels, whereby the resorption and the formation of new bone result not only as a front of healthy bone, but occur throughout the entire defect.

[0026] Due to the pore structure, the developed material is excellently suitable to take up the body's own osteo-inductive components (e.g., bone-marrow fluid) or those that are foreign to the body (e.g., BMPs). An extremely good tissue compatibility and thus a rapid ingrowing of bone tissue is achieved in this way.

[0027] The loose crystal structure makes possible a resorption by osteoclasts.

[0028] As calcium phospate, a hydroxyapatite is used preferably, which is adapted in crystallite size to biological apatite. A second soluble calcium phosphate component (β-tricalcium phosphate or brushite) can be selected as a local supplier of calcium phosphate for the biomineralization that begins at the collagen fibers. The soluble components will be present in such a concentration that either no resorptive inflammation will occur or only a small amount of resorptive inflammation will take place, which will not hinder the new formation of tissue.

[0029] In the literature, the positive influence of SiO_2 on collagen and bone formation has been increasingly reported [21-26].

[0030] The results were obtained both in in-vitro as well as in in-vivo experiments.

[0031] Carlisle [21] reported that silicon is an important trace element in the formation and mineralization of bone. A defective bone integration is produced in chickens and rats due to a deficiency of silicon in animal experiments [22]. Silicon is used in different forms in the experiments by different authors. Keeting et al. [23] used Zeolite A containing silicon for their experiments and established a positive effect on cell growth and cell division of cultured cells of a human cell line. Of course, it should be noted also that other elements, such as, e.g., aluminum, have a negative effect in the system.

[0032] The effect of silicon on bone formation was investigated in vitro on cell lines by Reffitt et al. [24]. A stimulation of type I collagen synthesis was established.

[0033] The loss of bone mass of osteoporotic rats was investigated in animal experiments [25]. In this way, it was established that rats which received 500 mg of Si per kg of diet showed no loss of bone mass, in contrast to that found in animals which had no Si in the diet. With in-vitro experiments, Lyu [26] established that Si plays a significant role in osteogenesis and a correlation exists between osteogenesis activity and Si concentration (from 10 to 100'pm* of Si in the culture medium).

[0034] The positive influence of SiO_2 during bone formation will be utilized in the described bone replacement material, by incorporating nanoporous SiO_2 in the loose crystal structure of the bone replacement material. Nanoporous SiO_2 is selected in order to produce, first of all, a good solubility, and secondly, to produce a large internal surface area.

---

* sic; ppm—Trans. Note.

Example 1

[0035] Fig. 1 shows a transmission-electron micrograph of sections of the biomaterial embedded in epoxide. The smooth surfaces are pores filled with epoxide. The loose crystal structure, which can be influenced by different calcium phosphate powders with different crystal morphology, can be clearly recognized. For this example, a ratio of 60% hydroxyapatite (HA) and 40% β-tricalcium phosphate (TCP) was selected for the calcium phosphate. The larger crystallites in the figure are the soluble β-TCP component.

[0036] The pore size is of the order of magnitude of the crystallites. Thus, a large surface area exists, which is wetted in vivo by body fluid.

[0037] The figure simultaneously demonstrates that pronounced interconnecting pores in the µm range are present (here, filled with epoxide due to the TEM preparation), which permit collagen fibers to grow in without hindrance.

[0038] The loose crystal structure which can be recognized here is achieved by using a molecular SiO_2 sol as a binder and that during drying, the SiO_2 molecules lie between the crystallites of the calcium phosphate and thus the crystallites are loosely joined with one another. Drying is conducted at temperatures of less than 400° Celsius, in order to prevent the sintering together of the calcium phosphate and the SiO_2 gel component. The SiO_2 is thus present as Xero gel. Fig. 1a shows an enlarged excerpt from Fig. 1. It can be clearly recognized that the calcium phosphate has a very loose crystal structure. The packing of the crystals is comparable to the arrangment of the calcium phosphate in natural bone. If the loose packing in the natural bone is produced by the collagen fibers lying between the crystallites, then the SiO_2 gel between the crystallites is responsible for the arrangement in the biomaterial.

[0039] Göttingen miniature pigs were used for the animal experiments. The

animals were adults (one year old) and weighed between 25 und 30 kg. The bone defects exceeded the critical size of 5 cm^3; their dimensions amounted to approximately 3.0 cm • 1.5 cm • 1.5 cm. The defects were placed in the lower jaw. These were then completely filled with the bone replacement material, and sealed with periosteum. After 5 weeks, the pigs were killed and the jaws were removed and subjected to x-ray, histological and scanning-electron-microscope investigations. The animal experiments were evaluated after 5 weeks in order to study the initial phase of bone regeneration.

[0040] A good ossification can be detected in the edge region. Histological sections from the edge region document a very good bone formation. The biomaterial is ensheathed by young bone in places (Fig. 2).

[0041] Clear indications of resorption can be recognized after 5 weeks. The originally "round" material now has developed edges and corners and displays indentations, as are typical for osteoclast activities (Fig. 3). It can also be observed that the micrometer pores of the material are penetrated by organic material. The SEM micrographs confirm this impression. Fig. 4 is a scanning electron micrograph of a section from the middle of the defect and represents an enlarged segment. Collagen fibers, which clearly show a mineralization, pass through the micropores in the entire defect—even centrally, where the bone formation has still not progressed.

[0042] Fig. 5 shows a demineralized histological section (hematoxylin-eosin). It can be seen that the large pores of the biomaterial permit an ingrowth of blood vessels, beginning from the edge.

Example 2

[0035] Fig. 6 shows a transmission-electron micrograph of sections of the biomaterial embedded in epoxide. The smooth surfaces are again pores filled

with epoxide. The loose crystal structure, which differs from that in Fig. 1, can be clearly recognized. For this example, pure hydroxyapatite (HA) was used as the calcium phosphate.

[0044] The pore size is of the order of magnitude of the crystallites. Thus, a large surface area exists, which is wetted in vivo by body fluid.

[0045] The figure simultaneously demonstrates that pronounced interconnecting pores in the µm range are present (here, filled with epoxide due to the TEM preparation), which permit collagen fibers to grow in without hindrance.

[0046] Figs. 6a to 6c show enlarged excerpts from Fig. 6. As in Example 1, the loose crystal structure is also produced here by SiO_2 molecules that lie between the calcium phosphate crystallites. Further treatment is conducted as in Example 1. The looser arrangement of the calcium phosphate crystallites is also comparable here with the arrangement of calcium phosphate crystallites in natural bone.

References

[1] Fox, R.: New bone. The Lancet 339, 463f. (1992)
[2] Kenley et al.: Biotechnology and bone graft substitutes. Pharmaceut. Res. 10, 1393 (1993)
[3] Reuter, F., Kübler, N. R.: The reconstruction of the lower jaw. Dtsch. Ärzteblatt 96 A, 1054ff. (1996)
[4] Kübler, N. R.: Osteo-induction and repair. Mund Kiefer Gesichts Chir. 1, 2ff. (1997)
[5] Yuan, H. et al.: Biomaterials 12, 1283ff. (2000) in press
[6] Wippermann, B. et al.: The influence of hydroxyapatite granules on the healing of a segmental defect filled with autologous bone marrow. Ann. Chir. Gynaecol. 88, 194ff. (1999)

[7] Anselme, K. et al.: Associations of porous hydroxyapatite and bone marrow cells for bone regeneration. Bone 25 (Suppl. 2), 5lSff. (1999)

[8] Niedhart, C. et al.: BMP-2 in injectable tricalcium phosphate carrier in the rat model is biomechanically superior to autologous spongy-bone plastics. Z. Orthop. 137 (Suppl. I), VI-283 (1999)

[9] Penel, G. et al.: Raman microspectrometry studies of brushite cement: in vivo evolution in a sheep model. Bone 25 (Suppl. 2), 8lSff. (1999)

[10] Brown, G. D. et al.: Hydroxyapatite cement implant for regeneration of periodontal osseous defects in humans. J Periodontol 69(2), 146ff (1998)

[11] Flautre, B. et al.: Volume effect on biological properties of a calcium phosphate hydraulic cement: experimental study in sheep. Bone 25 (Suppl. 2), 35Sff. (1999)

[12] Jansson, V. et al.: Bone/cartilage regeneration in bioimplants – Results of an animal exeriment study. Z. Orthop. 137 (Suppl. D), VI-307 (1999)

[13] Boyde, A. et al.: Osteoconduction in a large macroporous hydroxyapatite ceramic implant: evidence for a complementary integration and disintegration mechanism. Bone 24, 579ff. (1999)

[14] Meraw, S. J. et al.: Treatment of peri-implant defects with combination growth factor cement. J Periodontol 71 (1), 8ff. (2000)

[15] Glimcher, M. J.: Recent studies of the mineral phase in bone and its possible linkage to the organic matrix by protein-bound phosphate bonds. Philos Trans R Soc Lond B Biol Sci. 1984 Feb 13; 304(1121): 479-508.

[16] Cuisinier, F.; Bres E. F.; Hemmerle, J.; Voegel, J. C.; Frank, R. M.: Transmission electron microscopy of lattice planes in human alveolar bone apatite crystals. Calcif Tissue Int. 1987 Jun; 40(6): 332-8.

[17] Parry, D. A. The molecular and fibrillar structure of collagen and its relationship to the mechanical properties of connective tissue. Biophys Chem. 1988 Feb; 29(1-2): 195-209. Review.

[18] Arsenault, A. L.: Crystal-collagen relationships in calcified turkey leg tendons visualized by selected-area dark field electron microscopy. Calcif Tissue Int.

1988 Oct; 43(4): 202-12.

[19] Traub, W.; Arad, T.; Weiner, S.: Origin of mineral crystal growth in collagen fibrils. Matrix. 1992 Aug; 12(4): 251-5.

[20] Landis, W. J.; Hodgens, K. J.; Song, M. J.; Arena, J.; Kiyonaga, S.; Marko, M.; Owen, C., McEwen, B. F.: Mineralization of collagen may occur on fibril surfaces: evidence from conventional and high-voltage electron microscopy and three-dimensional imaging. J Struct Biol. 1996 Jul-Aug; 117(1): 24-35.

[21] E. M. Carlisle: A possible factor in bone calcification, Science 167, pp. 279-280 (1970).

[22] E. M. Carlisle: In vivo requirement for silicon inarticular cartilage and connective tissue formation in the chick, J. Nutr. 106, pp. 478-484 (1976)

[23] P. E. Keeting et al.: Zeolite A increases proliferation, differentation, and transforming growth factor β production in normal adult human osteoblast-like cells in vitro, J. of bone and mineral research, Vol. 7, No. 11, pp. 1281-1289 (1992)

[24] D. Reffitt et al.: Silicon stimulated collagen type I synthesis in human osteoblast-like cells, Bone 23(5), p. 419 (1998)

[25] H. Rico et al.: Effect of silicon supplement on osteopenia induced by ovariectomy in rats, Calcif. Tissue Int. 66(1), pp. 53ff (2000)

[26] K. Lyu, D. Nathason, L. Chou: Induced osteogenesity in vitro upon composition and concentration of silicon, calcium, and phosphorus. Sixth World Biomaterials Congress Transactions 2000, 1387

Patent Claims

1. An inorganic, resorbable bone replacement material based on calcium phosphate **is hereby characterized** in that:

a) a loose crystal structure of hydroxyapatite with interconnecting pores in the nanometer range is present between the crystallites, and the crystals, whose size is adapted to biological apatite, are joined only via a few groups of molecules, so that the volume fractions that are occupied by

collagen in natural bone are interconnecting pores,

b) the bone replacement material is permeated with interconnecting pores of the order of magnitude of 1 µm to 10 µm, which make possible the ingrowting of collagen fibers and

c) the bone replacement material contains additional interconnecting pores, which are appropriate for spongy bone, are of the order of 100 µm to 1000 µm and make possible the growing in of blood vessels.

2. The inorganic bone replacement material according to claim 1, further characterized in that it is comprised of hydroxyapatite and a soluble calcium phosphate, that a rapid biomineralization of the collagen bundle growing into the micrometer pores is initiated by this solubility, and is present in a concentration which does not give rise to resorptive inflammation that inhibits new formation of tissue.

3. The inorganic bone replacement material according to claims 1 and 2, further characterized in that nanoporous SiO_2 is incorporated in the loose crystal structure.

4. The inorganic bone replacement material according to claims 1 and 3, further characterized in that the internal surface is covered by synthetic growth factors or those intrinsic to the organism.

5 page(s) of drawings attached hereto

Drawings page 1

Number: **DE 100 60,036 C1**
Int. Cl.[7]:A **61 L 27/12**
Date published: August 8, 2002

Figures
Abb = Fig.

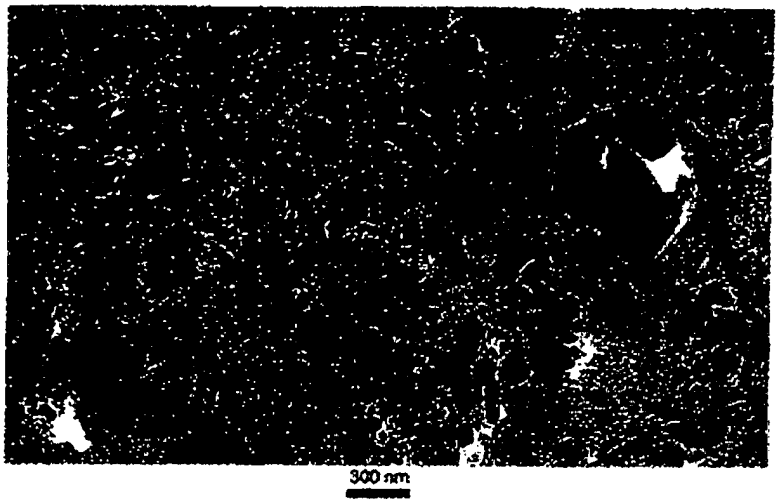

Abb. 1:

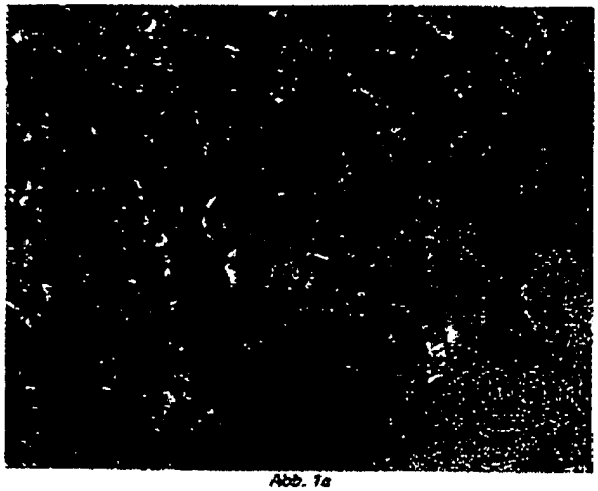

Abb. 1a

Drawings page 2

Number: **DE 100 60,036 C1**
Int. Cl.[7]:**A 61 L 27/12**
Date published: August 8, 2002

Abb = Fig.

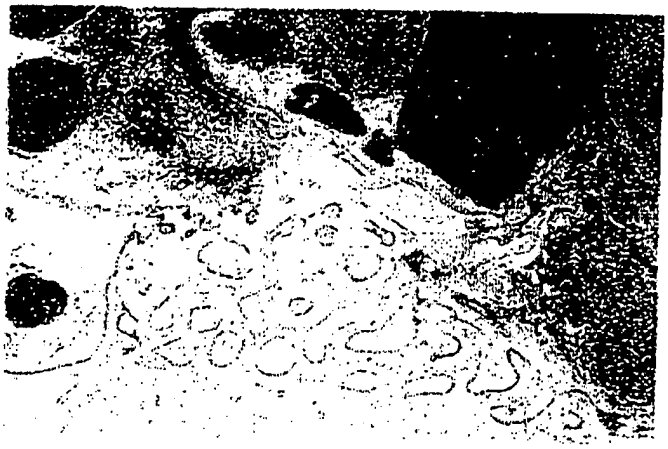

Abb. 2

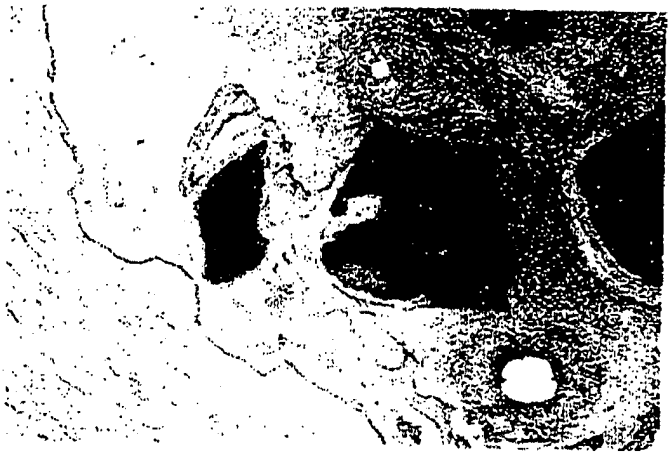

Abb. 3

Drawings page 3

Number: **DE 100 60,036 C1**
Int. Cl.[7]:**A 61 L 27/12**
Date published: August 8, 2002

Abb = Fig.

Abb.4

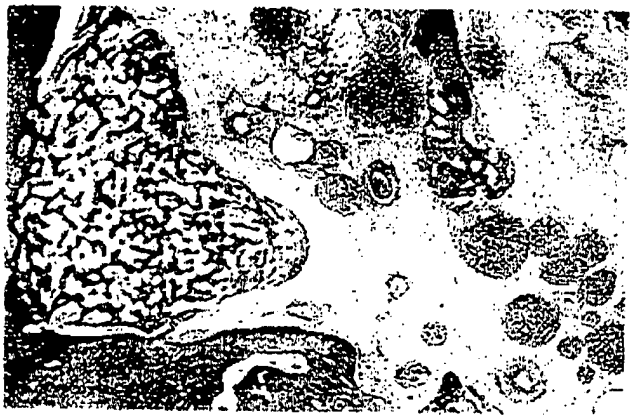

Abb. 5

Drawings page 4

Number: **DE 100 60,036 C1**
Int. Cl.[7]:**A 61 L 27/12**
Date published: August 8, 2002

Abb = Fig.

200 nm

Abb.6

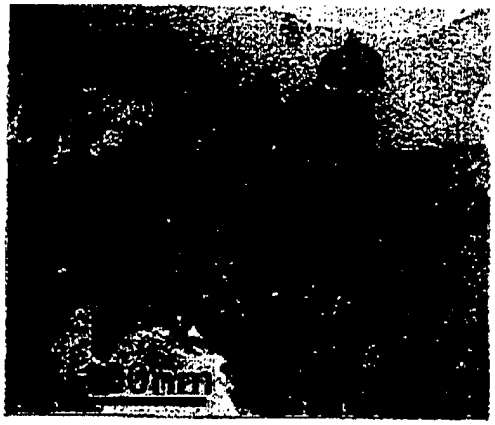

Abb. 6a

Drawings page 5

Number: **DE 100 60,036 C1**
Int. Cl.[7]:**A 61 L 27/12**
Date published: August 8. 2002

Abb = Fig.

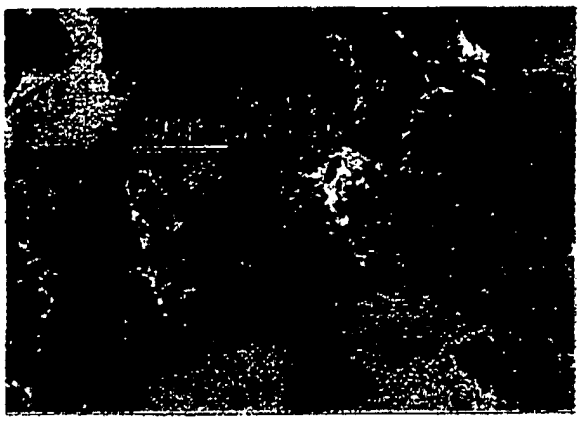

Abb. 6b

Abb. 6c